Empirical Nonparametric Bootstrap Strategies in Quantitative Trait Loci Mapping: Conditioning on the Genetic Model

Claude M. Lebreton* and Peter M. Visscher†

**John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, United Kingdom, and †Institute of Ecology and Resource Management, University of Edinburgh, Edinburgh EH9 3JG, Scotland, United Kingdom*

Manuscript received July 7, 1997
Accepted for publication October 8, 1997

ABSTRACT

Several nonparametric bootstrap methods are tested to obtain better confidence intervals for the quantitative trait loci (QTL) positions, *i.e.*, with minimal width and unbiased coverage probability. Two selective resampling schemes are proposed as a means of conditioning the bootstrap on the number of genetic factors in our model inferred from the original data. The selection is based on criteria related to the estimated number of genetic factors, and only the retained bootstrapped samples will contribute a value to the empirically estimated distribution of the QTL position estimate. These schemes are compared with a nonselective scheme across a range of simple configurations of one QTL on a one-chromosome genome. In particular, the effect of the chromosome length and the relative position of the QTL are examined for a given experimental power, which determines the confidence interval size. With the test protocol used, it appears that the selective resampling schemes are either unbiased or least biased when the QTL is situated near the middle of the chromosome. When the QTL is closer to one end, the likelihood curve of its position along the chromosome becomes truncated, and the nonselective scheme then performs better inasmuch as the percentage of estimated confidence intervals that actually contain the real QTL's position is closer to expectation. The nonselective method, however, produces larger confidence intervals. Hence, we advocate use of the selective methods, regardless of the QTL position along the chromosome (to reduce confidence interval sizes), but we leave the problem open as to how the method should be altered to take into account the bias of the original estimate of the QTL's position.

ONE aim of crop and animal physiologists is to elucidate how complex processes are regulated and integrated to achieve measurable production traits. The advent of molecular genetic markers has allowed quantitative geneticists to demonstrate that, even for complex traits such as tomato fruit size and composition (Paterson *et al.*, 1988) or crop yield (Edwards *et al.*, 1987, 1992; Stuber *et al.*, 1987), a small number of major genetic factors may explain a large proportion of the total genetic variance. Therefore, the validity of different models of causal relationships can, in theory, be compared on the basis of the identity of the genes that regulate different traits.

The approach of the quantitative geneticist does not allow the direct identification of the genes, but their positions can be estimated with the help of genetic markers. These positions are termed quantitative trait loci (QTLs). Molecular biologists can evaluate candidate genes by testing whether a QTL corresponds to the expression of a particular gene on the genome map whose primary product has been identified.

Corresponding author: Claude M. Lebreton, John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, UK.
E-mail: claude.lebreton@bbsrc.ac.uk

Whether comparing the QTLs for several traits or using the candidate gene approach, we have to contend with the uncertainty attached to the estimate of a locus position, and so, the comparison between the different models becomes a statistical test. Thus, for a single QTL, the ability to define a confidence interval around its estimated position is essential for testing the alternative hypotheses of close linkage *vs.* pleiotropy. The test statistic involved, however, may not follow a straightforward density of probability function.

LOD score–based or likelihood ratio–based methods were used by Lander and Botstein (1989) to define an approximation of a confidence interval on the estimate of a QTL's position, which they termed a "support interval." Jiang and Zeng (1995) also used likelihood ratios to test hypotheses such as close linkage *vs.* pleiotropy. These tests, however, are based on the assumption that the statistics cited above follow some asymptotic χ^2 distribution, which does not hold if the QTL is of small or medium effect in the framework of interval mapping, as demonstrated by van Ooijen (1992) and Mangin *et al.* (1994). Hence, in practice, the actual drop-off needed in the likelihood ratio statistic to define such support intervals would vary with each study and QTL. The problem becomes even more complicated when other markers are fitted in the mod-

els in addition to the markers flanking the interval being scanned, as in the "composite interval mapping" of Zeng (1993, 1994) and Basten *et al.* (1996) or in the "multiple QTL models" of Jansen (1993) and Jansen and Stam (1994). In particular, when these additional markers are situated on the chromosome being scanned, one can observe sharp falls in the likelihood ratio curve at some marker loci. This is caused by a change in the set of parameters present in the model and does not obviously correspond to a sudden decrease from one marker interval to the other in the probability of a QTL being at the position tested. Assuming the QTL exists, an estimated curve of density of probability of the QTL's position would be preferable. Visscher *et al.* (1996) approached this concept in an empirical way, by bootstrapping the original data and then examining the distribution of the estimates of the QTL's position. They simulated simple configurations of a chromosome with one QTL midway between two adjacent markers at positions 55 or 15 cM on a 100-cM map. The bootstrap procedure seemed a good alternative to the LOD drop-off for defining confidence intervals of estimates of the QTL position, but tended to be too conservative when the QTL accounted for less than 10% of the total trait variance.

In this study, we introduce selection of the bootstrapped samples as a means of conditioning our confidence interval estimates on the inferred number of distinguishable genetic factors. In so doing, we show that we significantly reduce the width of the estimated confidence intervals.

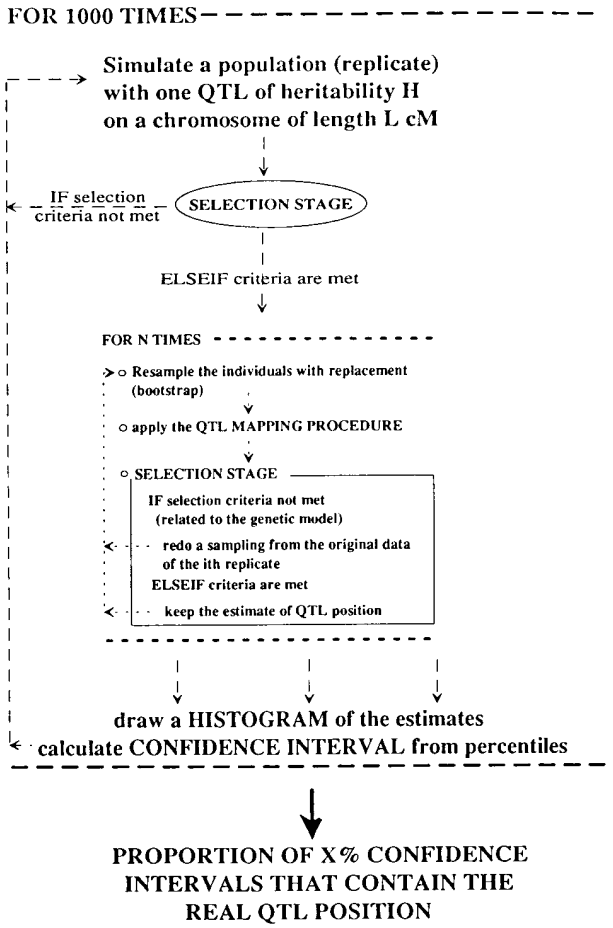

Figure 1.—Description of our general protocol to test the selective bootstraps.

MATERIAL AND METHODS

Simulation protocol: The protocol follows that presented by Visscher *et al.* (1996) and is summarized in Figure 1. Several bootstrap methods were compared for the same set of genetic parameters. The respective biases of the different methods and their divergence from one another were examined. In contrast to the work presented in Visscher *et al.* (1996), we explored the influence of the chromosome length. The role of the QTL's "centrality" was also examined (*i.e.*, whether in the middle or closer to one end of the chromosome). We focused only on recombinant populations from crosses between inbred lines. For each genome configuration tested, 1000 backcross populations were simulated, which from now on we will call "replicates" to remain homogenous with the terminology of Visscher *et al.* (1996).

Two series of simulations were carried out: The first series intended to examine a broad range of issues involved in the selection step of the selective bootstraps. Two selective and a nonselective empirical bootstrap procedures were compared. The marker and trait data were resampled jointly with replacement. In the selective bootstraps, the selection was based on criteria related to the estimated number of genetic factors, and only the retained bootstrap samples contributed a value to the empirically estimated distribution of the QTL position estimate. Symmetrical confidence intervals of X% were calculated by taking the $[(100 - X)/2]$th and the $[X + (100 - X)/2]$th percentiles of the bootstrap-estimated distribution of the QTL position as their lower and upper limits, respectively. We investigated the proportion of zero-, one-, two-, and three-QTL outcomes over the simulated populations and bootstrap samples. This allowed us to check whether the rejection of the outcomes with zero and more than one QTL, given their respective proportions, had a significant effect on the size of the confidence intervals and their conservativeness compared to just rejecting cases with zero QTL. Thus, we devised the following two sorts of selective bootstraps: (1) a "selective bootstrap" version 1, where we only retained the resampled data sets that showed the same number of QTLs on the chromosome of interest and the same sign for the QTL of interest as were observed in the original data set, *i.e.*, one QTL with a positive additive effect in our simulations, and (2) a "selective bootstrap" version 2, where we retained the resampled data and fitted one and only one QTL whenever there was a significant effect present along the chromosome of interest, *i.e.*, whatever the observed number of significant QTLs along the chromosome provided that a significant effect was detected. In our "non-selective bootstrap," as many QTLs as the number observed from the original data set were fitted, irrespective of significance or sign, *i.e.*, even when no significant effect was detected along the chromosome.

Unlike Visscher *et al.* (1996), who applied the bootstrap either on every replicate or on replicates with a significant effect present along the chromosome only (*i.e.*, with one or more QTLs), in this series of simulations, the bootstraps were only applied to the replicates that yielded one and only one QTL on the chromosome. Apart from this difference, as far as

the bootstrap itself is concerned, our nonselective one is similar to that implemented by Visscher *et al.* (1996) in the case when a single QTL is simulated.

In this first series of simulations, we also examined the effects of chromosome length and QTL position on the divergence between the two sorts of selective bootstraps described above.

The second series of simulations was more focused on determining precisely, *i.e.*, without any bias, the percentage of inclusion of the real QTL position in the estimated confidence intervals to check if the selective bootstrap was actually providing inclusion percentages closer to the target values. Because we resorted to a different QTL mapping method in this second series, as explained below, the nonselective bootstrap was only compared to selective bootstrap version 2. Also, the bootstraps were applied on replicates that showed a significant effect present along the chromosome (*i.e.*, not only on those that showed one QTL only).

The genomes were made of only one chromosome for simplicity. To generate the simulated genotypes, Haldane's formula was used to translate genetic distances into recombination frequencies to be compatible with the QTL mapping procedures we used (described below). Environmental residuals were normally distributed.

QTL mapping methods: The QTL mapping procedure is called as many times as the data are resampled, so its computation time was the factor to minimize in both series of simulations. To keep the computation fast while addressing two different priorities involved in the two series of simulation, namely precise estimation of the number of QTLs in the first one and precise estimation of the QTL position in the second one, we had to resort to two different QTL mapping procedures.

First QTL mapping procedure: In the first QTL mapping procedure, which we shall designate as "marker selection method," we implemented the principles established by Stam (1991), Zeng (1993), Rodolphe and Lefort (1993), and Wright and Mowers (1994), which state that the partial regression coefficient of marker i only depends on the QTLs located between marker $(i - 1)$ and marker $(i + 1)$. Given a sufficient marker density throughout the genome, we can overlook the fact that the within-marker type trait distributions are joint distributions caused by the recombination between the marker(s) and the QTL. One can then assume no significant loss of QTL detection power. Thus, we need to only carry out a selection of the "best" subset of marker regressors from over the whole covered genome. Then, we can infer the minimum number of QTLs solely from the number of markers selected, their position relative to each other (whether adjacent or not), and the sign of their respective partial regression coefficients. This way, we can decide to reject a bootstrapped sample on the basis of the inferred model without having lost any time in calculating the precise parameter estimates of its QTLs.

Our implementation of this logic consisted of the following three stages: (1) selection of a best subset of marker regressors, (2) translation of the subset into a QTL model, and (3) refinement of the QTL parameter estimates.

Multiple linear models with fixed effects were used to regress the trait phenotypic values onto the marker scores. Since standard statistical methods were used, the programming of this method was performed in the standard statistical package Genstat 5, release 3.1 (Genstat 5 Committee, 1993). The three stages are described below.

At the marker selection stage, a first, "artificially" lax forward procedure, with an inclusion F-ratio of 4.0, added a subset of all the markers of the chromosome; some of which were included by chance, because of the lax inclusion ratio. Then, a stringent backward procedure was implemented to reject some markers. The level of stringency adopted in the backward procedure corresponded to the 5% genome-wise (and therefore, chromosome-wise) type I error worked out empirically by permutations as in Churchill and Doerge (1994). In these permutations, a forward procedure only was implemented on each permuted sample and the observed F-statistic from the inclusion of the most significant marker in the model was stored. The top 5th percentile of this F-statistic distribution determined our exclusion threshold for a 5% genome-wise type I error.

The forward-backward succession was preferred to fitting all the markers of the chromosome, and then applying a backward selection (although the latter maximizes the QTL detection power) because of potential problems of collinearity or pseudo-collinearity between the marker-regressors that could arise. This happens for markers that are close to one another but distant marker-regressors can also be collinear or pseudo-collinear as a result of chance when there are many. This would have generated a higher error in the parameter estimates and in the set of parameters that were finally retained. A second reason for preferring a prior forward selection was a gain in computing time because the maximum number of regressors fitted was lower.

For a given exclusion F-ratio, varying the value of the F (lax) inclusion ratio does not alter the QTL detection power. However, it affects the respective proportions of one-, two-, n-... QTL outcomes. Detecting two QTLs when there is only one is a type I error. Thus, although it decreases the power to detect a second QTL by 50%, compared to fitting all the markers in a first stage, as suggested above, a value of 4.0 for the inclusion F-ratio allowed us to keep the "type I erroneous" detection of a second QTL close to 5% on average in the bootstrapped samples as described in results. This percentage was fairly stable over the different chromosome lengths tested and, therefore, the inclusion F-ratio was kept at 4.0 for all the configurations. This target figure of 5% was chosen arbitrarily.

In the interpretation stage (the second stage), when isolated markers were selected during the first stage, *i.e.*, separated from one another by nonselected ones, the number of QTLs was assumed to be the number of selected markers. When two adjacent markers were selected, if the partial regression coefficients of these two markers were of the same sign, one QTL was assumed within the interval. If the partial regression coefficients were of opposite signs, two QTLs were assumed. If n $(n > 2)$ adjacent markers are selected, $n - 1$ QTLs were assumed.

Calculation of the QTL parameter estimates: For any population size, there is a certain marker density beyond which the power to detect a QTL is not significantly increased. When this density is reached, one can observe that the confidence interval on a QTL position spans several marker intervals. Therefore, for the sake of computation speed, in the design of the QTL mapping procedure, stress was not put on the precise location of the QTL within a marker interval.

Thus, when an isolated marker was selected during the first stage, the QTL was fitted at the marker locus, and its additive effect was assumed to be the partial regression coefficient of that marker. This compromise is one among many possible compromises, and it is certainly not optimal, but it offers very significant gains in computation speed. Only when two adjacent markers were selected, and if the partial regression coefficients of these two markers were of the same sign, a QTL was fitted within the interval according to the following formulas (derived in the appendix section):

$$D_{j,k} = 1/4 \ln \beta_{j+1} - 1/4 \ln \beta_j + \tfrac{1}{2} D_{j,j+1} \qquad (1)$$

$$\boldsymbol{a} = \exp\left(\tfrac{1}{2} \ln \beta_j + \tfrac{1}{2} \ln \beta_{j+1} + D_{j,j+1}\right), \quad (2)$$

where $D_{j,k}$ represents the estimated distance in centimorgans between the marker "on the left" and the QTL, $\boldsymbol{a}$ represents the estimated additive effect of the QTL, β_j, β_{j+1} represents the partial regression coefficients of the trait phenotype on the marker type, the markers j and $j + 1$ being fitted in turn, and D_{jj+1} represents the distance in centimorgans between the flanking markers (j and $j + 1$).

Compared to the related method of Whittaker *et al.* (1996), our method fits only one marker of the interval in turn. This alleviates the problem of pseudocollinearity between these markers, which would otherwise affect the accuracy of the partial regression coefficient estimates if the flanking markers are close to each other, *i.e.*, 2 or 3 cM, given the small population sizes we investigated. If more than two adjacent markers with partial regression of the same sign are selected, the linear approximation shows its limitations compared to a maximum likelihood-based method. The case is simply insoluble if we only use the observed additive effect at each marker, because, for example, when three adjacent markers are retained, we have four parameters to estimate (two QTL positions and their two effects) from only three statistics (the three partial regression coefficients). It is unlikely, however, that three adjacent markers will be retained in practice or in our simulated configurations of one QTL per chromosome.

Second QTL mapping procedure: The second series of simulations were analyzed with the program described in Visscher *et al.* (1996). It was amended slightly to perform a "selective bootstrap" similar to version 2 of the first series of simulations, as well as the nonselective one, but carries out the linearized interval mapping by applying the equations of Whittaker *et al.* (1996) and only fits a one QTL model on the chromosome. All the successive pairs of adjacent markers were tested in turn, and in the nonselective bootstrap, the pair providing the smallest residual sum of squares was retained to fit the QTL whether or not the *F*-ratio from the comparison of the full and reduced models was significant. In the selective bootstrap, only the outcomes with a significant *F*-ratio were retained. For this second series, the programs were written in FORTRAN 77.

The genomic configurations simulated: In the first series, the heritability and the population size were set so as to get a realistic experimental power [$(1-\beta)$; β being the type II error of missing a real QTL on the chromosome] while keeping the population size small. This was to check whether the sampling with replacement scheme was robust when applied to small samples for the QTL position estimate. Hence, we chose a relatively high heritability for a single QTL: 10% and simulated populations of 70 backcross individuals only. One hundred and fifty bootstraps were first carried out to define a confidence interval. The simulated chromosome lengths were 100, 140, and 180 cM, again to remain realistic. The QTL was simulated in the middle of the chromosome, except for the longest chromosome, where it was also simulated at 15 cM from one end.

We noticed that on most bootstrapped samples, pairs of adjacent markers were less frequently selected than on the original replicate data. More often, the markers retained in the model were isolated. Therefore, the positioning of the QTLs over the bootstrapped samples was discrete, which in turn made the lower and upper limits of the confidence intervals take less accurate values. As a consequence, to increase the resolution of the test protocol, a compromise had to be reached between accuracy of QTL positioning and speed of computation in the context of our simulations. Over the many bootstrapped samples, the estimated QTL positions more likely appear to be near the real one. Therefore, when we compared the percentages of inclusion of the real QTL position by the estimated confidence intervals, we set up a gradually increasing density of markers around the real QTL position, *i.e.*, up to one marker every 2.5 cM, but only one marker every 40 cM at the ends of the chromosome (Figure 2). The power of detection was thus significantly decreased toward the ends, but it did not affect the comparison between the different methods in qualitative terms, *i.e.*, which one was more conservative than the other.

In the second series of simulations, we explored two population sizes, 70 and 200 backcross individuals, two chromosome lengths, 100 and 180 cM, and a set of QTL positions

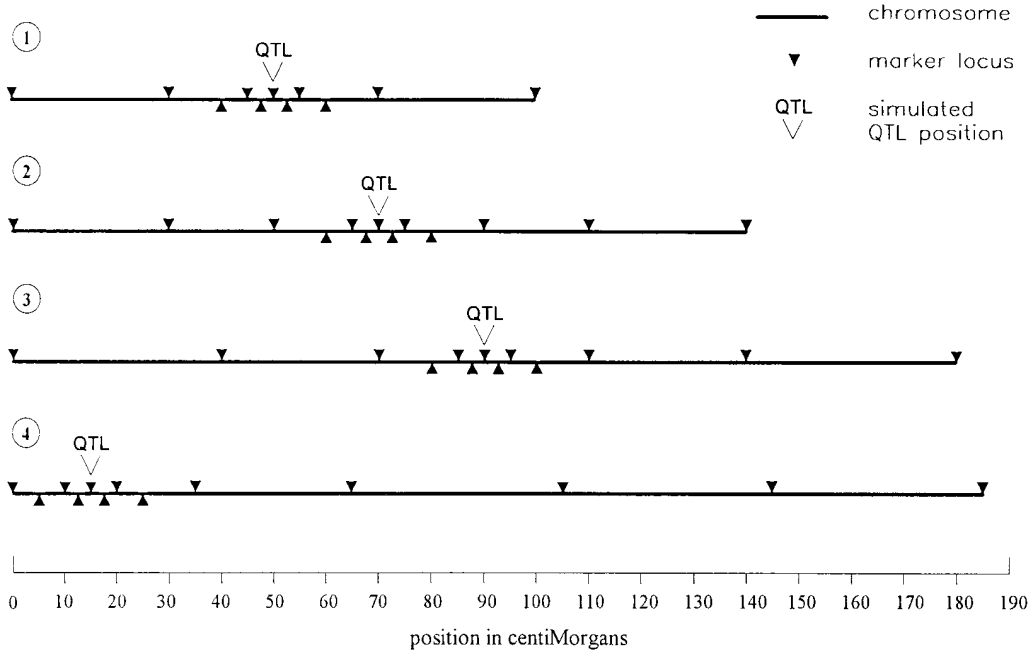

Figure 2.—Presentation of the four marker configurations in the first set of simulations.

along the chromosome ranging from 5 cM from the first marker up to a central position. The length and the conservativeness of confidence intervals of different stringencies (50–95%) were estimated. The markers were regularly spaced every 10 cM. The QTL positions simulated were always exactly in the center of the intervals to avoid any confounding effect caused by a variation in detection power within the interval (G. A. Walling, P. M. Visscher, and C. S. Haley, unpublished results). The heritability of the QTL was adjusted to maintain approximately the same experimental power for the two population sizes. This was achieved according to the following formula in Soller *et al.* (1976):

$$h_2^2 = y/(y+1) \text{ with } y = (N_1/N_2)h_1^2/(1-h_1^2), \qquad (3)$$

where N_1 and N_2 are the population sizes 1 and 2, respectively, and h_1^2 and h_2^2 are the corresponding heritabilities to maintain the same detection power.

RESULTS

From the first series of simulations: The results of 1000 simulated populations of 70 individuals, using the "marker selection method," are presented in Table 1 alongside results from the same configurations, but using the method of Whittaker *et al.* (1996). The genome was made of a 180-cM long chromosome with markers evenly spaced every 10 cM. To obtain a chromosome-wise type I error of 5%, the same *F*-ratio of 8.85 was applied in both methods. The resulting QTL detection powers did not significantly differ between the two methods and were ~50%. The QTL position did not have any apparent effect either on the detection power. The number of two-QTL cases are below 5%, and the percentage of outcomes with more than two QTLs is minute. The respective proportions of estimated numbers of QTLs for the bootstrapped samples, taken from the selected replicates, were significantly different from the ones observed on the original replicates. The proportion of cases with one and two QTLs increased at the detriment of the other cases. The proportion of two-QTL cases, however, does not go beyond 6%.

Table 2 shows the results of 1000 simulated populations of 70 individuals, using the marker selection method, for several genomic configurations and an uneven marker density as described above. The exclusion *F*-ratio ranges from 7.17 for configuration 1 to 8.4 for configurations 3 and 4, as they are named in the table, so as to maintain a genome-wise type I error of 5%. The percentages of estimated number of QTLs from the original replicates does not vary significantly across the genomic configurations. Compared to the previous experiment, the QTL detection power has decreased significantly because of the lower marker density away from the simulated QTL position, and it lies only between 30 and 35%. The proportion of cases with at least two QTLs has also significantly decreased and does not go beyond 3.5%. However, the respective proportions of estimated numbers of QTLs for the bootstrapped samples taken from the selected replicates are very similar to those from the previous experiment with the markers regularly spaced. Also, they remain remarkably stable over the four genomic configurations.

A 95% confidence interval was calculated both with the nonselective bootstrap described above and with the selective bootstrap version 1. Over the different simulated populations, the percentages of confidence intervals containing the real QTL position were compared. Their difference was tested by pairwise comparisons because the bootstraps were applied to the same sets of replicates. The results are presented in Table 3.

TABLE 1

Comparison of the percentages of *n* QTL outcomes between the two QTL mapping methods

QTL mapping method	Position	Percentage of *n* QTL outcomes				CI width
		0 QTL	1 QTL	2 QTLs	3 QTLs	
Marker selection period	85	51.8 ± 3.1	41.9 ± 3.1	4.9 ± 1.4	1.1 ± 0.7	81.3 ± 3.8 cM
		14.8 ± 1.1	**79 ± 1.2**	**5.8 ± 0.6**	**0.4 ± 0.1**	
	15	53.6 ± 3.1	41.6 ± 3.1	3.8 ± 1.2	0.9 ± 0.6	76.2 ± 3.8 cM
		14.7 ± 1.1	**78.7 ± 1.2**	**5.9 ± 0.6**	**0.6 ± 0.1**	
Whittaker *et al.* (1996)	85	50 ± 3.2	50 ± 3.2			92 cM ± 4 cM
		17 ± 1.1	**83 ± 1.1**			
	15	50 ± 3.2	50 ± 3.2			86 cM ± 4 cM
		17 ± 1.1	**83 ± 1.1**			

One thousand replicates of 70 backcross individuals were simulated with a 180-cM chromosome. One QTL of 10% heritability situated at 85 cM from one end. Markers were regularly spaced every 10 cM. In both methods, the exclusion of *F*-ratio was 8.85. The second column mentions the simulated QTL position.

For the second marker selection method: The percentages of zero-, one-, two-, and three-QTL outcomes over the 1000 replicates are represented in plain text. The percentages of zero-, one-, two-, and three-QTL outcomes from the 150 bootstraps per replicate averaged over the replicates are printed in bold. For the method Whittaker *et al.* (1996), the columns for the one-, two-, and three-QTL outcomes are fused since these cases cannot be distinguished by the method. The last column presents the average 95% confidence interval (CI) width in centimorgans.

TABLE 2

Results from the first set of simulations—effect of the chromosome length and the QTL position on the estimated QTL number derived from the first QTL mapping method

No.	Chromosome length	QTL position	Percentage of *n* QTL outcomes			
			0 QTL	1 QTL	2 QTLs	3 QTLs
1	100 cM	Central	65 ± 3	31 ± 3	3 ± 1	0.5 ± 0.4
			15.3 ± 1	**77.7 ± 1**	**6.6 ± 0.5**	**0.4 ± 0.1**
2	140 cM	Central	67.9 ± 3	29.7 ± 3	1.9 ± 1	0.4 ± 0.4
			15.4 ± 1	**77.6 ± 1**	**6.4 ± 0.5**	**0.4 ± 0.1**
3	180 cM	Central	66.6 ± 3	31.2 ± 3	1.8 ± 1	0.2 ± 0.2
			15.4 ± 1	**77.6 ± 1**	**6.5 ± 0.5**	**0.4 ± 0.1**
4	185 cM	15 cM	70.1 ± 3	27.1 ± 3	2.4 ± 1	0.3 ± 0.3
			15.7 ± 1	**77.6 ± 1**	**6.3 ± 0.5**	**0.4 ± 0.1**

One thousand replicates of 70 backcross individuals were simulated. The heritability of the QTL was 10%. Markers were densely spaced around the QTL position, every 2.5 cM, and gradually less densely toward the chromosome ends, every 30 or 40 cM, as shown in Figure 2. The exclusion *F*-ratio was 7.17 for configuration 1, 8 for configuration 2, and 8.4 for configurations 3 and 4, as they are named in the table, so as to maintain a genome-wise type I error of 5%. For each replicate that showed one and only one significant QTL, with the same sign as that simulated, 150 bootstraps were performed. In each of the four configurations, the QTL is located at a marker locus. The percentages of *n* QTL outcomes over the 1000 replicates are represented in plain text. The percentages of *n* QTL outcomes from the 150 bootstraps per replicate, averaged over the replicates, are printed in bold.

The divergence between the two methods and the rejection rate in the selective procedure seem to increase with the chromosome length and the QTL noncentrality; however, this apparent effect of the QTL noncentrality is not confirmed by the second set of simulations.

The average confidence interval size can be limited by the chromosome length itself; therefore, its comparison between the two methods is more representative for the longer chromosome in our case given the large values we obtain. Thus, in configuration 3, the nonselective procedure yielded an average confidence interval 94.3 cM wide, whereas for the selective one, it was "only" 63.5 cM wide. This difference was significant at $P < 0.001$. The nonselective method seems always too conservative. Although the noninclusion percentages are subjected to large errors, those from the selective bootstrap seem consistently closer to the expected 5% and, therefore, they seem less biased.

A pairwise comparison for the noninclusion percentages was then carried out to compare the selective bootstrap version 1 against the selective bootstrap version 2 in the configuration that most brings out differ-

TABLE 3

Results from the first set of simulations—comparison between selective bootstrap method version 1 and the nonselective one

No.	Chromosome length	QTL position	Noninclusion %		*P*	Average CI width in cM	
			Select.	Nonselect.		Select.	Nonselect.
1	100 cM	Central	5.3 ± 1.7	4.3 ± 2	0.021	41	48
2	140 cM	Central	3.7 ± 2.4	2.0 ± 2.4	0.011	54	72
3	180 cM	Central	3.6 ± 1.5	2.6 ± 2.6	0.006	63	94
4	185 cM	15 cM	5.2 ± 2.5	3.1 ± 2.5	0.002	74	114

One thousand replicates of 70 backcross individuals were simulated. The heritability of the QTL was 10%. Markers were densely spaced around the QTL position, every 2.5 cM, and gradually less densely toward the chromosome ends, every 30 or 40 cM (same experiment as in Table 2). The first column mentions the configuration number as referred to in the text.

In the fourth column, the subcolumn on the left is the percentage of noninclusion of the real QTL position in the estimated confidence interval from the selective bootstrap version 1 with its confidence limits. The subcolumn on the right is obtained with the nonselective bootstrap. *P* is the probability that the two methods yield the same proportions. The next two columns contain the average confidence interval widths in centimorgans for the selective and nonselective bootstraps, respectively.

Select., value obtained with selective bootstrap version 1; Nonselect., value obtained with nonselective bootstrap.

ences between the different bootstrap strategies, *i.e.*, configuration 4. The comparison yielded a probability of 0.12 (result not shown elsewhere) for the null hypothesis that the two bootstrap methods give similar noninclusion percentages, which is not low enough to reject it. Thus, no significant differences could be found for the noninclusion percentages between the two. Therefore, version 2 can be considered as an acceptable approximation of version 1 for bootstrapping in cases when only one QTL is detected from the original data, the case on which we focus exclusively in this paper.

TABLE 4

Second set of simulations—long chromosome, small population size[a]

POS.	SEL.	E(P)→	50	60	70	80	85	90	95
5	No sel.	O(P)→	35	44	53	63	69	75	85
		CI	57	72	87	107	118	131	148
	Sel.	O(P)→	43	51	59	68	74	79	85
		CI	19	26	35	47	55	66	84
15	No sel.	O(P)→	47	58	65	77	85	89	97
		CI	55	68	83	101	114	126	147
	Sel.	O(P)→	45	53	61	75	80	85	92
		CI	20	27	36	49	57	68	86
25	No sel.	O(P)→	49	59	71	81	85	90	95
		CI	50	63	78	97	109	122	142
	Sel.	O(P)→	43	52	62	74	79	86	92
		CI	20	28	36	47	54	65	81
35	No sel.	O(P)→	53	64	74	84	90	93	97
		CI	45	58	73	93	104	119	139
	Sel.	O(P)→	42	53	62	72	79	85	93
		CI	20	28	37	47	55	65	82
55	No sel.	O(P)→	60	69	80	88	91	95	98
		CI	45	56	71	89	101	116	136
	Sel.	O(P)→	46	54	63	76	81	86	92
		CI	22	29	38	48	55	65	78
75	No sel.	O(P)→	60	71	80	89	93	96	99
		CI	41	52	66	85	97	113	135
	Sel.	O(P)→	43	53	63	74	79	84	92
		CI	20	26	34	45	52	63	81
85	No sel.	O(P)→	59	71	79	87	93	96	99
		CI	39	52	66	83	98	112	139
	Sel.	O(P)→	44	54	66	78	84	90	95
		CI	21	27	36	48	57	70	92

[a] Comparison between the selective (presence of effect on the chromosome as a whole and sign of effect) bootstrap method and the nonselective one for different QTL positions.

Results from 1000 replicates of the following configuration: genome made up of one chromosome 180 cM long with markers evenly spaced (one every 10 cM), one QTL of 10% heritability, a population of 70 backcross individuals, and selection of the replicates and bootstrapped samples on the basis of an *F*-ratio threshold of 8.85 and on the sign of the QTL effect.

POS., distance between the "first" marker and the QTL simulated; Sel., whether we applied the selective bootstrap or not; E(P), percentage of confidence intervals that contain the real QTL position; O(P), percentage of confidence intervals that contain the real QTL position; CI, size of the confidence intervals in centimorgans.

From the second series of simulations: In this series of experiments, presented in Tables 4–7, the power of the experiment was ~50% because of the regular spacing of the markers—every 10 cM for all the genomic configurations tested, as shown above. Although the two bootstrap methods were not applied to the same series of populations with this protocol, and therefore could not be compared through a pairwise procedure, it was still possible to detect some significant divergences over the large number of replicates analyzed. The observed values for the 50% confidence intervals vary within ±4.3%, whereas for the 95% confidence intervals, they vary within ±2% only (percentages not shown elsewhere).

Table 4 shows the results from 1000 replicates of the following configuration. The genome consisted of one chromosome, 180 cM long, one QTL of heritability 10%, and a population of 70 backcross individuals. The selection of the replicates and of the bootstrapped samples was based on an *F*-ratio threshold of 8.85 and on the sign of the QTL effect.

The confidence interval sizes remained constant over the different QTL positions for both methods. Apart from the chromosome length itself, which sets an upper limit, the power of the experiment was the only parameter that defined the confidence interval size for a given bootstrap method. It is interesting to note that the nonselective method generated confidence intervals on the QTL position that were almost twice as large as those for the selective one: 140 *vs.* 80 cM. When the QTL was centrally situated, the selective method seemed to offer a definite improvement. While the nonselective method was consistently very conservative over the whole range of confidence interval stringencies, the selective one was only slightly anticonservative for the low stringencies and became unbiased for the 90% and 95% confidence intervals.

When the QTL heritability increased for this same configuration, however, the power of the experiment became close to 100%, and both methods converged toward being anticonservative, as is shown in the first row of data in Table 5. Also, when the QTL was not centrally situated in Table 4, the selective bootstrap seemed relatively and consistently conservative, whereas the nonselective one was consistently anticonservative to the same extent. On average, for example, 92% of the 95% confidence intervals generated by the selective method contained the real QTL position compared with 97% with the nonselective method. At a level of noncentrality corresponding to positions ~15–25 cM from the first marker in the configuration tested, the nonselective method seemed the least biased, if biased at all, across the range of confidence interval stringencies. When the QTL was situated very close to the end of the chromosome, both methods seemed consistently anticonservative.

The configurations in Table 6 differ from those in

TABLE 5

Second set of simulations—long chromosome, "big" population size[a]

Pop. size	H2	Selection	E(P)	50	60	70	80	85	90	95
70	0.50	No & yes	O(P)	45	54	64	74	81	85	93
200	0.0374	No	O(P)	64	74	84	91	94	97	99
200	0.0374	Yes	O(P)	43	52	63	76	82	89	95

[a] Comparison between the selective (presence of effect on the chromosome as a whole and sign of effect) bootstrap method and the nonselective one for different QTL positions.

Results from 1000 replicates of the same configuration as in Table 2, except that the population size is 200. This set was intended to explore the effect of the population size, the experimental power remaining constant, and the effect of an increase in detection power with the population size remaining constant. Therefore, only the central position of the QTL was simulated. In the first row, the results from the selective and nonselective procedures are featured together because they do not differ because of the high experimental power.

H2, heritability of the simulated QTL; E(P), percentage of confidence intervals that contain the real QTL position; O(P), percentage of confidence intervals that contain the real QTL position.

TABLE 6

Second set of simulations—short chromosome, small population size[a]

POS.	SEL.	E(P)→	50	60	70	80	85	90	95
5	No sel.	O(P)→	42	51	59	71	76	83	90
		CI size:	30	38	46	56	63	70	80
	Sel.	O(P)→	40	47	59	68	73	78	87
		CI size:	14	18	24	31	36	42	52
45	No sel.	O(P)→	57	67	79	88	92	94	98
		CI size:	24	30	39	50	56	65	76
	Sel.	O(P)→	44	53	62	73	80	86	92
		CI size:	16	21	26	34	38	44	54

[a] Comparison between the selective (presence of effect on the chromosome as a whole and sign of effect) bootstrap method and the nonselective one for different QTL positions.

Results from 1000 replicates of the following configuration: genome made up of one chromosome 100 cM long with markers evenly spaced (one every 10 cM), one QTL of 10% heritability, a population of 70-backcross individuals, and selection of the replicates and bootstrapped samples on the basis of an *F*-ratio threshold of 7.37 and on the sign of the QTL effect.

POS, distance between the "first" marker and the QTL simulated; SEL, whether we applied the selective bootstrap or not; E(P), percentage of confidence intervals that contain the real QTL position; O(P), percentage of confidence intervals that contain the real QTL position; CI size, size of the confidence intervals in centimorgans.

Table 4 only by the chromosome length, which is now 100 cM long. Table 7 is very similar to Table 6, but the population size is 200. The values for the confidence interval sizes and the proportions of inclusion are very similar. When the QTL was very close to one end of the chromosome, both methods seemed anticonservative, but the nonselective one less so. When the QTL is rather centrally situated, the nonselective method is conservative and the selective one is anticonservative by the same extent in terms of percentage of inclusion. This similarity of the results between Tables 6 and Table 7 shows that the population size *per se* (*i.e.*, for a constant detection power) does not affect the behavior of the bootstraps and, therefore, does not bring any confounding effect in the comparison between the selective and the nonselective bootstrap.

DISCUSSION

We have simulated simplistic cases of only one QTL on a single chromosome with an additive effect and no dominance or epistasis. This was to examine whether selection during the resampling improved the bootstrap procedure compared to the nonselective one, which was conservative over most QTL configurations.

The choice of our two selective resampling methods was by no means arbitrary or *ad hoc*. On the contrary, the choice of our selective method version 1, was motivated by the following reasoning: (1) From the definition of the bootstrap (see Efron and Tibshirani 1993), it would seem natural to apply the same rules on the resampled data as on the original data to calculate the statistic of interest, whose distribution we want to estimate. (2) When retaining the position of a QTL detected from the original data, in the first instance, we implicitly assume that the QTL does exist, *i.e.*, we condition our position estimate on the fact that there exists a QTL. Indeed, had we not detected a significant QTL from the original data, we would not have kept the (most likely) position estimate (in the full model tested). (3) It would therefore seem *a priori* legitimate to apply the same rule during the bootstrapping and only retain position estimates when the QTL effect appears significant.

A prerequisite to resampling data from a finite size sample (our replicate), however, is that the raw data matrix constitutes an unbiased sample from which the bootstrap parameter estimates asymptotically follow the real distribution of the parameter (the QTL position). Because a minimum significance threshold is imposed in our case, the expected estimated value of a QTL effect is biased upward in absolute value, as documented by Hyne *et al.* (1995). Because QTLs of larger effect have smaller confidence intervals, the prerequisite is therefore not respected. This would intuitively make a case against reapplying on the resampled data the same procedure as on the original replicates, *i.e.*, including a selection step. On the other hand, however, there is nothing in the bootstrap theory that justifies not applying any selection at all on the bootstrap replicates to compensate for a bias caused, precisely, by selection of

TABLE 7

Second set of simulations—Short chromosome, "big" population size[a]

POS.	SEL.	E(P)→	50	60	70	80	85	90	95
5	No sel.	O(P)→	42	50	60	71	78	84	92
		CI size:	30	38	46	57	63	71	81
	Sel.	O(P)→	39	53	64	78	78	83	89
		CI size:	16	20	25	32	37	43	53
45	No sel.	O(P)→	59	70	80	87	91	95	98
		CI size:	24	30	38	49	56	64	76
	Sel.	O(P)→	44	56	64	76	82	88	94
		CI size:	16	21	26	34	38	45	56

[a] Comparison between the selective (presence of effect on the chromosome as a whole and sign of effect) bootstrap method and the nonselective one for different QTL positions.

Results from 1000 replicates of the same configuration as in Table 4, except that the population size is 200. To keep the same experimental power, the heritability of the QTL was 3.74%. Selection of the replicates and of the bootstrapped samples was on the basis of an *F*-ratio threshold of 7.70 and on the sign of the QTL effect.

POS, distance between the "first" marker and the QTL simulated; SEL, whether we applied the selective bootstrap or not; E(P), percentage of confidence intervals that contain the real QTL position; O(P), percentage of confidence intervals that contain the real QTL position; CI, size of the confidence intervals in centimorgans.

the original replicates. As we did not find the means to address the issue in an analytical way, we resorted to numerical simulations to check whether, compared to no selection step at all, the benefits of inserting the previous selection step in the bootstrap procedure outweighed the bias caused by applying the bootstrap on biased samples. Our selective method, version 2, was meant to constitute an approximation to the previous version that is quicker to run and applicable on a wider range of QTL mapping methods.

The simulations showed that when calculating symmetrical confidence intervals, none of the tested bootstrap methods remained consistently the best overall. One or the other performed better, depending on the relative position of the QTL along the chromosome. This rather inconvenient property may be explained by the truncation of the distribution of the density of the probability of a QTL's detected position when this QTL is actually situated close to one end of the chromosome. Thus, when a QTL is detected, the estimator of its position is biased toward bringing it closer to the center of the chromosome, as demonstrated by Hyne *et al.* (1995). Even an ideal, unbiased confidence interval, if applied on a biased estimate, will appear anticonservative. In the absence of bias on the estimation of the QTL's position, *i.e.*, when the QTL is actually situated centrally, the selective bootstrap method seems to be an unbiased estimator of a confidence interval. When the estimation of the QTL's position becomes biased, *i.e.*, when the QTL is situated closer to one end of the chromosome, the conservativeness of the nonselective method compensates the initial bias. In this latter case, however, because we know the bias, although 95% of the 95% confidence intervals contain the QTL, the average width of the confidence interval could be reduced by still implementing a selective resampling scheme that would take this bias into account. Another means of reducing the confidence interval width when the QTL is close to one end of the chromosome would be to take their upper and lower limits in a nonsymmetrical way from the selective bootstrap empirical distribution. For example, one could retain the bottom value of the distribution as the lower limit and the 95th percentile as the upper limit, instead of the 2.5th and the 97.5th percentiles, respectively. Indeed, picking two points off the empirical distribution of the estimated QTL position notably looses much of the relevant information about shape, as conveyed by the bootstrap histogram (DiCiccio and Efron 1996). Thus, a postbootstrap analysis could consist of comparing the widths of the confidence intervals obtained by varying a parameter α from $-X$ to $+X$ and retaining the narrowest one, 100-X being the coverage percentage of the interval, and $(X + \alpha)/2$ and $100 - (X - \alpha)/2$ being their lower and upper percentile limits, respectively. Further theoretical developments will be needed to achieve this, and the problem is left as an open question to the reader.

We have also seen that when the power of the experiment increases, the two methods converge and are relatively anticonservative, as shown in Table 5. The convergence can be explained by the increasingly low proportion of bootstrapped samples that fail to find a QTL, hence, a very low rejection rate by the selective bootstrap, thus making little difference to the nonselective bootstrap. Incidentally, it is important to emphasize that in our simulations, the 90% of unexplained variance is of environmental origin. This is different from the case where there would be several chromosomes bearing other QTLs. Our QTL would then be one among other QTLs, and part of the background noise would be removable. The confidence intervals on the QTL parameters would then be smaller. The observed general anticonservativeness in this case, given the small size of the confidence intervals, is actually caused by some within-interval variation in detection power, as studied by G. A. Walling, P. M. Visscher and C. S. Haley (unpublished results). Thus, to check this hypothesis, we simulated the same configuration, again with the QTL centrally situated, but at a marker locus this time. Both bootstrap methods were then conservative (*e.g.*, 97% of the 95% confidence intervals contain the QTL). But this within-interval variation constitutes a different subject matter to the comparison of bootstrapping methods, and it is relevant only when we are dealing with small confidence intervals, *i.e.*, because of high experimental powers or the choice of a low stringency (*e.g.*, a 50% confidence interval as opposed to a

95% confidence interval). Incidentally, this explains why the 50% intervals in Tables 4–7 often appear anticonservative, even when the corresponding 95% intervals look unbiased.

We did not investigate cases of more than one QTL on a chromosome because of the confounding effects that would arise from the difficulty in detecting two QTLs simulated in coupling, unless they are some considerable distance apart. Another confounding factor is the bias of a QTL position estimate resulting from the proximity of another QTL.

The estimation of a QTL position from the marker and phenotypic data is not a straightforward process. In our case, multiple linear models are involved and, furthermore, the variables fitted differ from one bootstrap sample to the other. Thus, the effect of the sample size alone on the quality of the bootstrap is difficult to predict. Some statistics that are based on the tails of the empirical distribution, such as our confidence intervals, are very sensitive to problems of smoothness (Efron and Tibshirani 1993). The smoothness obtained from sample sizes as small as 70 might, *a priori*, have affected the quality of the percentile method *per se* to define a confidence interval and brought some confounding effect to our comparison between resampling schemes. From our comparison between sample sizes of 70 and 200, while keeping the experimental power constant by adjusting the trait heritability, it turns out that the percentile method used to define confidence intervals is robust even for sizes as small as 70 backcross individuals because the confidence interval sizes are similar to those with 200 individuals.

In conclusion, although the biases are relatively small for the different bootstrap methods we examined, with commonly found experimental powers of the order of 50% to detect a particular QTL, a selective bootstrap significantly reduces the size of the confidence interval on its position estimate, compared to the nonselective bootstrap presented in an earlier paper, without affecting its coverage probability. Further reduction in the confidence interval size could possibly be achieved, when a QTL is detected near a telomere, by using a bias-corrected, selective, nonparametric bootstrap and also by implementing the percentile method in a nonsymmetrical way. The sizes of the confidence intervals obtained and their biases are good enough for marker-assisted selection, but far from sufficient for cloning or fine-scale mapping.

We are grateful to the Association Xavier Bonnemaizon, Lescar, France, to the British Council for funding the first year of the corresponding author's Ph.D. project, and to the John Innes Foundation for funding the second year of the same Ph.D. project. We also thank Roger Payne and Peter Lane for providing us with their invaluable expertise in writing the Genstat programs. We express our gratitude to M. J. Kearsey, J. Brown, S. A. Quarrie, John Flintham, Chris Haley, and Grant Walling for their helpful comments on the contents of this manuscript, and Robin Thompson for useful discussions on the subject.

LITERATURE CITED

Basten, C. J., Z.-B. Zeng and B. S. Weir, 1996 QTLCartographer: a suite of programs for mapping quantitative trait loci, p. 108 in *Abstracts to Plant Genome IV.* Scherago International Inc., New York.

Churchill, G. A., and R. W. Doerge, 1994 Empirical threshold values for quantitative trait mapping. Genetics **138:** 963–971.

DiCiccio, T. J., and B. Efron, 1996 Bootstrap confidence intervals. Stat. Sci. **11:** 189–228.

Edwards, M. D., C. W. Stuber and J. F. Wendel, 1987 Molecular marker-facilitated investigations of quantitative trait loci in maize. I. Numbers, genomic distribution and types of gene action. Genetics **116:** 113–125.

Edwards, M. D., T. Helentjaris, S. Wright and C. W. Stuber, 1992 Molecular facilitated investigations of quantitative trait loci in maize. IV. Analysis based on genome saturation with isozyme and restriction fragment length polymorphism markers. Theor. Appl. Genet. **83:** 765–774.

Efron, B., and R. J. Tibshirani, 1993 *An Introduction to the Bootstrap*. Chapman & Hall, New York.

Genstat 5 Committee, 1993 *Genstat™ 5 Release 3 Reference Manual*, Clarendon Press, Oxford.

Hyne, V., M. J. Kearsey, D. J. Pike and J. W. Snape, 1995 QTL analysis: unreliability and bias in estimation procedures. Mol. Breed. **1:** 273–282.

Jansen, R. C., 1993 Interval mapping of multiple quantitative trait loci. Genetics **135:** 205–211.

Jansen, R. C., and P. Stam, 1994 High resolution of quantitative traits into multiple loci via interval mapping. Genetics **136:** 1447–1455.

Jiang, C., and Z.-B. Zeng, 1995 Multiple trait analysis of genetic mapping for quantitative trait loci. Genetics **140:** 1111–1127.

Lander, E. S., and D. Botstein, 1989 Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics **121:** 184–199.

Mangin, B., B. Goffinet and A. Rebai, 1994 Constructing confidence intervals for QTL location. Genetics **138:** 1301–1308.

Paterson, A. H., E. S. Lander, J. D. Ewitt, S. Peterson, S. E. Lincoln *et al.*, 1988 Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature **335:** 721–726.

Rodolphe, F., and M. Lefort, 1993 A multi-marker model for detecting chromosomal segments displaying QTL activity. Genetics **134:** 1277–1288.

Soller, M., T. Brody and M. A. Genizi, 1976 On the power of experimental designs for the detection of linkage between marker loci and quantitative loci in crosses between inbred lines. Theor. Appl. Genet. **47:** 35–39.

Stam, P., 1991 Some aspects of QTL mapping, pp. 23–32. *Proceedings of the Eighth Meeting of the Eucarpia Section Biometrics in Plant Breeding*. Brno, Czech Republic.

Stuber, C. W., M.D. Edwards and J.F. Wendel, 1987 Molecular marker-facilitated investigations of quantitative trait loci in maize. II. Factors influencing yield and its component traits. Crop Sci. **27:** 639–648.

Visscher, P. M., R. Thompson and C.S. Haley, 1996 Confidence intervals in QTL mapping by bootstrapping. Genetics **143:** 1013–1020.

van Ooijen, J. W., 1992 Accuracy of mapping quantitative trait loci in autogamous species. Theor. Appl. Genet. **84:** 803–811.

Whittaker, J. C., R. Thompson and P. M. Visscher, 1996 On the mapping of QTL by regression of phenotype on marker-type. Heredity **77:** 23–32.

Wright, A. J., and R. P. Mowers, 1994 Multiple regression for molecular-marker, quantitative trait data from large F2 populations. Theor. Appl. Genet. **89:** 305–312.

Wu, W. R., and W. M. Li, 1996 Model fitting and model testing in the method of joint mapping of quantitative trait loci. Theor. Appl. Genet. **92:** 477–482.

Zeng, Z.-B., 1993 Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. Proc. Natl. Acad. Sci. USA **90:** 10972–10976.

Zeng, Z.-B., 1994 Precision of quantitative trait loci. Genetics **136:** 1457–1468.

Communicating editor: Z-B. Zeng

APPENDIX:

Calculation of the QTL parameters: Assuming a QTL of additive effect a and dominance effect d, flanked by markers j and $j + 1$, with meiotic or observed recombination frequencies of $R_{j,k}$ between the QTL and marker j and $R_{j+1,k}$ between the QTL and marker $j + 1$, we have the following:

$$\beta_j = a(1 - 2\ R_{j,k}) \text{ and } \beta_{j+1} = a(1 - 2\ R_{j+1,k}), \quad \text{(A1)}$$

the expectations of the regression coefficients of the phenotype on the marker types obtained by regressing phenotypes onto markers one at a time. If we then choose to use Haldane's function to convert $R_{j,k}$ and $R_{j+1,k}$ into $D_{j,k}$ and $D_{j+1,k}$, the corresponding distances in Morgans, β_j and β_{j+1}, in the case of F_2s, backcross populations (BC), doubled haploid lines (DHL), can then be expressed as follows:

$$\beta_j = a \exp(-2\ D_{j,k}) \text{ and } \beta_{j+1} = a \exp(-2\ D_{j+1,k}). \quad \text{(A2)}$$

Equations A2 can then be rewritten as a system of two equations with two unknowns by carrying out this logarithmic transformation:

$$\ln(\beta_j) = \mathbf{ln}\ \boldsymbol{a} - \mathbf{2}\boldsymbol{D_{j,k}} \quad \text{(A3)}$$

$$\ln(\beta_{j+1}) = \mathbf{ln}\ \boldsymbol{a} + 2\boldsymbol{D_{j,k}} - 2D_{j,j+1} \quad (D_{j,k} + D_{j+1,k} = D_{j,j+1}). \quad \text{(A4)}$$

Solving the system we obtain the following:

$$\boldsymbol{D}_{j,k} = 1/4 \ln \beta_{j+1} - 1/4 \ln \beta_j + \tfrac{1}{2}\ D_{j,j+1} \quad \text{(A5)}$$

$$\boldsymbol{a} = \exp(\tfrac{1}{2} \ln \beta_j + \tfrac{1}{2} \ln \beta_{j+1} + D_{j,j+1}) \quad \text{(A6)}$$

These formulas are used to calculate $D_{j,k}$ and a by replacing the βs by the absolute values of their observed values and by reestablishing the right sign of a according to the sign of the βs. Likewise, d, the dominance effect in the case of an F_2 population, is calculated according to a formula similar to a, but the βs are replaced by regression coefficients of the trait on dummy variables calculated as $VARIATE_{DOM} = 1 - |VARIATE_{ADD}|$ if $VARIATE_{ADD}$ corresponds to the allelic dosage minus one as described in Whittaker *et al.* (1996) (-1, 0, $+1$ for *mm*, *mM*, and *MM*, respectively).

In the case of recombinant inbred lines (RIL), because $R_{j,k}$ and $R_{j+1,k}$ are observed recombination frequencies (as opposed to single meiosis recombination frequencies), the transformation cannot be achieved in a format as convenient as in A2, which would lead to a linear system of equations. Nevertheless, Wu and Li (1996) demonstrated that using the Kosambi metric to work out the D_s (genetic distances), A1 can then again be expressed as A2. This is why we construct the genetic map assuming no interference, *i.e.*, using Haldane's metric in the case of BC, DHL, and F_2s, but would assume some and use Kosambi's metric for RIL.